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ORIGINAL ARTICLE

Prognostic value of pretreatment plasma D-dimer level in dogs with intermediate to high-grade non-Hodgkin lymphoma

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Abstract

Pretreatment D-dimer levels have been reported to predict survival in several types of malignancies in human patients. The objective of this study was to evaluate the prognostic value of pretreatment D-dimer level in dogs with intermediate to high-grade non-Hodgkin lymphoma (NHL). In a prospective, randomized, double-blind study of F14512 vs etoposide phosphate, we assessed the prognostic value of pretreatment plasma D-dimer level in 48 client-owned dogs diagnosed with intermediate to high-grade NHL. The correlation between pretreatment plasma D-dimer level and various clinical features, progression-free survival (PFS) and overall survival (OS) was analysed. The median value of pretreatment plasma D-dimer level was 0.4 μg/mL (range: 0.1-14.3 μg/mL). High pretreatment plasma D-dimer level (>0.5 μg/mL) was detected in 44% (21/48) of dogs. High D-dimer levels were not correlated with naive vs relapsed lymphoma, clinical stage, substage, immunophenotype or treatment group. D-dimer levels >0.5 μg/mL were significantly associated with inferior median PFS (54 vs 104 days, P = .011) and OS (93 vs 169 days, P = .003). In the multivariate analysis, high D-dimer levels remained an independent predictor for worse PFS (HR: 3.21, 95% CI: 1.57-6.56, P = .001) and OS (HR: 3.87, 95% CI: 1.88-7.98; P < .001). This study suggests that pretreatment plasma D-dimer level can serve as a predictor of prognosis in dogs with intermediate to high-grade NHL. Further studies are warranted to confirm these findings.

KEYWORDS

D-dimer, dog, lymphoma, prognostic marker, survival

1 | INTRODUCTION

Non-Hodgkin lymphomas (NHL) are among the most common haematopoietic cancers in both human and dog populations. Canine NHL shares many biological and therapeutic similarities with their human counterpart, including clinical presentation, biological behaviour and therapeutic responses.[1-3] Many clinical and biological factors have prognostic value in the duration of remission and survival in dogs with NHL, including WHO clinical stage,[4,5] and substage,[4,6,7] immunophenotype,[4,8-12] histopathological grade,[4,11,13] cytomorphological subtype,[14,15] anatomical location,[16-18] and prior steroid treatment.[11,19] Other potential prognostic markers have been evaluated, such as argyrophilic nucleolar organizing regions (AgNOR staining),[20] Ki-67 antibody staining,[21] serum lactate dehydrogenase

(LDH) activity,[22,23] serum C-reactive protein,[24,25] and serum thymidine kinase 1 (sTK1) activity[26] but the accuracy of these markers remains controversial. Therefore, it is important to determine simple and accurate prognostic factors for risk stratification before treatment initiation.

D-dimer, a stable end-product of fibrin degradation, is a widely used and highly sensitive global indicator of activated coagulation and fibrinolysis.[27] Elevated levels of D-dimer have been reported to correlate with poor prognosis in several types of malignancies in human patients, including breast,[28] colorectal,[29,30] lung,[31-33] gastric,[34,35] gynaecological cancers,[36] and diffuse large B-cell lymphoma.[37] The mechanisms underlying the prognostic value of D-dimers in cancer patients are not fully understood. The tumour cells or the stimulation of tumour-associated inflammatory cells can activate the clotting cascade and the activation of coagulation and fibrinolysis and may play an important role in tumour growth, infiltration, metastasis and angiogenesis.[38,39] As a compensatory mechanism for fibrin clot formation, fibrinolysis is activated, and then D-dimer is produced.[40-42] Higher plasma D-dimer levels have been identified in dogs with malignant neoplasia compared with dogs with benign disease,[43] and plasma D-dimer levels have been reported to be associated with advanced tumour stage[44] and high-grade tumour.[45,46] However, the clinical significance of D-dimer levels in dogs with NHL has rarely been investigated. Therefore, the objective of this study was to determine the correlation between pretreatment plasma D-dimer levels with clinical features and survival in dogs with spontaneously occurring intermediate to high-grade NHL.

2 | MATERIALS AND METHODS

2.1 | Patient selection

This study was part of a comparative oncology project assessing a new polyamine-vectorized anticancer drug (F14512) compared with the parent drug etoposide, in dogs with naturally occurring NHL.[47] The study design was prospective, randomized and double-blinded. The study design and the data analysis were carried out by OCR (Oncovet-Clinical-Research), Loos, France. The study was conducted at Oncovet, a private referral centre for small animal oncology, Villeneuve d'Ascq, France.

In the present study, client-owned dogs were considered eligible for inclusion if they (i) had a histologically and/or cytologically confirmed diagnosis of intermediate to high-grade NHL; (ii) had measurable disease at the time of inclusion (allowing staging and clinical response assessment); (iii) had no anticancer drugs in the month before inclusion (including steroids); and (iv) had no significant biochemical abnormalities or blood cytopenia, which would precluded the use of cytotoxic drugs. The exclusion criteria were: (i) active infection at the time of inclusion; (ii) a history of venous or arterial thromboembolism or anticoagulant treatment within the 3 months prior to inclusion; and (iii) any known congenital coagulative abnormality. Written informed consent form was obtained from the owner before enrolment of each dog.

2.2 | Initial staging

All dogs were staged based on the modified WHO five-stage criteria for canine lymphoma as previously described.[47] Initial staging tests included a complete blood count, blood smear evaluation, biochemistry panel, ionized calcemia, whole-body computed tomography, fine-needle aspirates (FNA) and biopsies of peripheral lymph nodes, ultrasound-guided liver and spleen FNA, urine analysis and bone marrow aspirates. Bone marrow was considered infiltrated if neoplastic lymphoid cells represented ≥3% of all nucleated cells on the basis of bone marrow cytology.[48] The definitive diagnosis was confirmed for each dog from cytological examination of the lymph node aspirates performed by one board-certified clinical pathologist (C. Fournel-Fleury) and lymphoma was graded according to the updated Kiel morphological classification.[49] Immunophenotyping was performed using immunohistochemistry as previously described.[50] The cytology results and immunophenotype were recorded for all dogs.

2.3 | Measurement of pretreatment plasma D-dimer levels

Fasting venous blood samples were collected before treatment initiation. Plasma D-dimer concentrations were measured using a turbidometric immunoassay (Nyco Card Reader II, NYCOMED) according to the manufacturer's instructions. The D-dimer reference range of 0.1 to 0.5 μg/mL was used based on published data in clinically healthy dogs.[51]

2.4 | Measurement of pretreatment sTK1 activity (DiviTum assay)

Serum samples were collected for sTK1 activity measurement prior to treatment initiation. Analysis of sTK1 activity was determined by a refined ELISA assay, the DiviTum assay (lab Biovica International, Uppsala, Sweden), according to the manufacturer's instructions (http://biovica.com/), as previously described in dogs.[52] The absorbance readings to DiviTum units per litre (Du/L) were converted using the values from standards with known TK activity, with a minimum detectable activity for this assay of 20 Du/L. The analysis was performed at the Biovica laboratory in Uppsala, Sweden.

2.5 | Treatment modality and follow-up

The treatment protocol used in this study has been previously described.[47] The same induction chemotherapy protocol was followed in all dogs over a period of 8 weeks. Dogs were randomly assigned to receive F14512 (0.075 mg/kg), or etoposide phosphate (100 mg/m^2), delivered over a 3-hour intravenous (IV) infusion once daily for three consecutive days, every 2 weeks for 4 cycles (ie, days 1-3, days 15-17, days 29-31, days 43-45). The maximum tolerated dose of F14512

and etoposide phosphate was previously evaluated using two independent traditional 3 + 3 phase I dose-escalation trials.[50,53]

The response to treatment was evaluated before each chemotherapy administration. The best response to treatment was determined based on physical examination and peripheral lymph node size measurement using a calliper, according to the VCOG Response Evaluation Criteria for Peripheral Nodal Lymphoma in dogs version 1.0.[54] At the end of 4 cycles (day 62), dogs underwent a complete end-staging.

After completion of the 4 cycles, dogs who experienced a clinical benefit (complete or partial response or stable disease) received three additional consolidation cycles with one IV injection of the same drug on a 3-week cycle (day 68, 89 and 110). Clinical follow-up was continued every 4 weeks from completion of treatment until disease progression, death or withdrawal from the study as a result of the owner's decision. In cases of disease progression or withdrawal, dogs were excluded and a doxorubicin-based chemotherapy protocol was offered to the dog's owner.

2.6 | Statistical analysis

The primary endpoints of the study were progression-free survival (PFS) and overall survival (OS). PFS was measured from randomization to any of the following events, whichever occurred first: progression, relapse, death (related to the lymphoma or not) and evaluated according to the VCOG criteria v1.0.[54] The OS was defined as the time from randomization to death from any cause, or to the time of the last follow-up assessment for dogs who remained alive. The survival data was calculated using the Kaplan-Meier method and compared using a log-rank test. Categorical variables were expressed as numbers and percentages, and groups were compared with the chi-square test or, for small numbers, Fisher exact test. Data was tested for normal distribution using Shapiro-Wilk test for normality. For continuous, normally distributed variables, data was expressed as mean and SD, and groups were compared with *t*-test or analysis of variance as appropriate. Non-normally distributed continuous variables were expressed as median and range, and groups were compared using Wilcoxon rank test or Krustal-Wallis test as appropriate. Evaluation of correlation between pretreatment plasma D-dimer levels and pretreatment sTK1 activity was performed using a Spearman correlation coefficient. The Cox proportional hazard model was used for a univariate screen of all potential predictors of survival. A univariate analysis was performed using the following six parameters: prior chemotherapy, WHO clinical stage, WHO clinical substage, immunophenotype, treatment group and pretreatment plasma D-dimer level. The *P* values of the six outcome univariate tests were corrected for multiple comparisons with false discovery rate (FDR) correction by the Benjamini & Hochberg method.[55] The variables with statistical significance were included in the multivariate analysis using a stepwise forward Cox regression model. Results were considered statistically significant with a *P* value <.05. All statistical analysis was performed with SPSS version 24.0 software (SPSS A, Inc., Chicago, Illinois).

2.7 | Cell line validation statement

No cell line validation testing has been conducted.

3 | RESULTS

3.1 | Epidemiological and clinical characteristics

Forty-eight dogs with naturally occurring intermediate to high-grade NHL were included in the randomized double-blind study between December 3, 2014 and August 30, 2016. All 48 dogs met the inclusion criteria and were analysed. Forty-one (85%) dogs had no prior treatment, and seven (15%) dogs were enrolled after relapse following a CHOP-based chemotherapy protocol. Twenty-five (52%) dogs were randomized to receive F14512 and 23 (48%) dogs to receive etoposide phosphate. The last day of follow-up was January 30, 2017. The main clinical characteristics are summarized in Table 1.

TABLE 1 Clinical characteristics of dogs with intermediate to high-grade non-Hodgkin lymphoma (n = 48)

Clinical characteristics	Dogs
Number of dogs (%)	48 (100%)
Sex, no. (%)	
Male	27 (56%)
Female	21 (44%)
Age, years, mean ± SD (range)	7.5 ± 2.7 (3-14)
Body weight, kg, mean ± SD (range)	28.5 ± 11.7 (8.0-51.4)
WHO clinical stage, no. (%)	
Stage II	2 (4%)
Stage III	8 (17%)
Stage IV	29 (60%)
Stage V	9 (19%)
WHO clinical substage, no. (%)	
Substage a	26 (54%)
Substage b	22 (46%)
Immunophenotype, no. (%)	
B-cell lymphoma	37 (77%)
T-cell lymphoma	7 (15%)
Unclassified	4 (8%)
Prior treatment, no. (%)	
Untreated	41 (85%)
Prior chemotherapy	7 (15%)
Treatment group, no. (%)	
F14512	25 (52%)
Etoposide phosphate	23 (48%)

Abbreviation: WHO, World Health Organization.

3.2 | Pretreatment plasma D-dimer level

For the entire cohort, the median value of pretreatment plasma D-dimer level was 0.4 μg/mL (range: 0.1-14.3). Twenty-seven (56%) dogs presented with a pretreatment plasma D-dimer level within the normal range (≤0.5 μg/mL), and 21 (44%) dogs had a pretreatment plasma D-dimer level above 0.5 μg/mL.

3.3 | Pretreatment plasma D-dimer level and clinical characteristics

Baseline clinical characteristics of the cohort are presented and compared between dogs with a low (≤0.5 μg/mL) or high (>0.5 μg/mL) pretreatment plasma D-dimer level in Table 2. There was no correlation between high pretreatment D-dimer level and clinical characteristics including sex, age, body weight, WHO clinical stage and substage, immunophenotype and prior chemotherapy received before inclusion. Treatment modalities were balanced between dogs with a low (≤0.5 μg/mL) or high (>0.5 μg/mL) pretreatment plasma D-dimer level (P = .99).

3.4 | Correlation between pretreatment plasma D-dimer level and pretreatment sTK1 activity level

Pretreatment sTK1 activity was measured in all dogs. The median sTK1 activity was 1374 Du/L (range: 20-60 005 Du/L). A low

TABLE 2 The clinical characteristics and outcome of dogs with low (≤0.5 μg/mL, n = 27) or high (>0.5 μg/mL, n = 21) pretreatment plasma D-dimer levels

Parameters	Number of dogs (%) with D-dimer ≤ 0.5 μg/mL	Number of dogs (%) with D-dimer > 0.5 μg/mL	*P* value
Overall, no. (%)	27 (100%)	21 (100%)	
Sex, no. (%)			0.25[a]
Male	13 (48%)	14 (67%)	
Female	14 (52%)	7 (33%)	
Age, years, median (range)	7 (3–13)	7 (4-14)	0.40[b]
Body weight, kg, mean ± SD (range)	26.8 ± 10.4 (11.4-51.4)	30.8 ± 13.1 (8-50)	0.26[c]
WHO clinical stage, no. (%)			0.15[a]
Stage II + III + IV	24 (89%)	15 (71%)	
Stage V	3 (11%)	6 (29%)	
WHO clinical substage, no. (%)			0.24[a]
Substage a	17 (63%)	9 (43%)	
Substage b	10 (37%)	12 (57%)	
Immunophenotype, no. (%)			0.44[a]
B-cell lymphoma	22 (81%)	15 (71%)	
T-cell lymphoma	3 (11%)	4 (19%)	
Prior chemotherapy, no. (%)			0.11[a]
Untreated	21 (78%)	20 (95%)	
Prior chemotherapy	6 (22%)	1 (5%)	
Treatment group, no. (%)			0.99[a]
F14512	14 (52%)	11 (52%)	
Etoposide phosphate	13 (48%)	10 (48%)	
Outcome			
ORR, no. (%)	21 (78%)	17 (81%)	0.99[a]
PFS, days (range)	104 (10–224)	54 (4-153)	0.011[d]
OS, days (range)	169 (10–466)	93 (4-280)	0.003[d]

Abbreviations: ORR, overall response rate (defined as complete response plus partial response); OS, overall survival; PFS, progression-free survival; WHO, World Health Organization.
[a]Fisher exact test.
[b]Wilcoxon test.
[c]*T* test.
[d]Log-rank test.

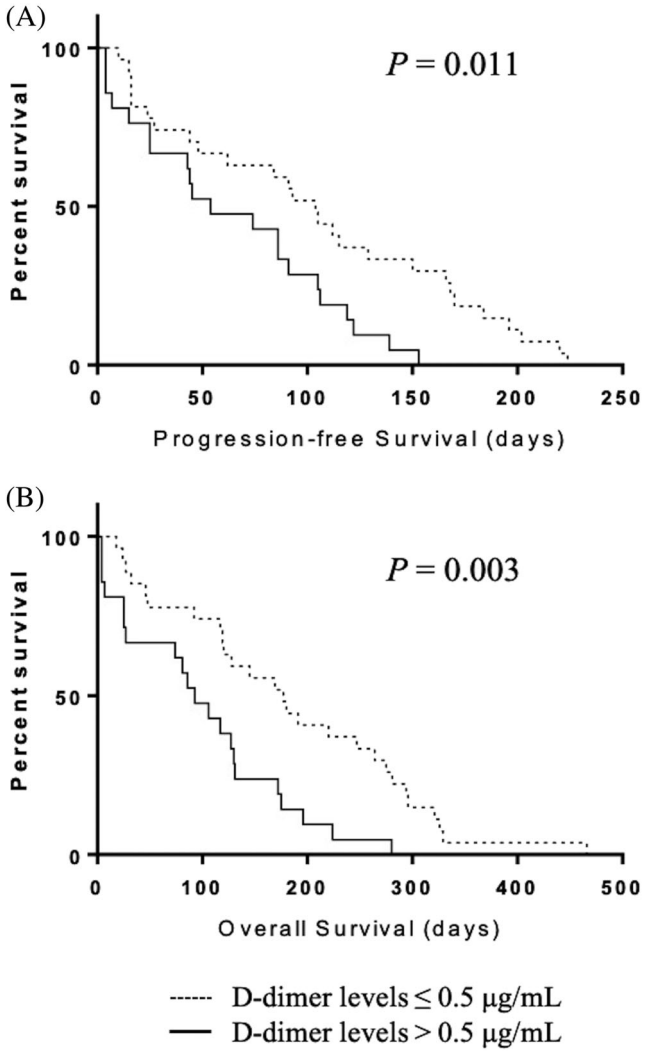

FIGURE 1 Kaplan-Meier survival curves of progression-free survival, A and overall survival, B, in dogs with high (>0.5 µg/mL, n = 21) or low (≤0.5 µg/mL, n = 27) pretreatment D-dimer level. Log-rank *P* values are shown

correlation was observed between pretreatment plasma D-dimer level and pretreatment sTK1 activity (Spearman correlation r_s = 0.38, 95% CI: 0.10-0.61, *P* = .007). Serum TK1 activity was not correlated with PFS (*P* = .16) or OS (*P* = .20); Supporting Information Figure S2.

3.5 | Pretreatment plasma D-dimer level and outcome

Thirty-eight (79%) dogs achieved an objective response with 21 complete response (CR) and 17 partial response (PR). No significant difference in response rate was observed between dogs with a pretreatment D-dimer level >0.5 µg/mL and dogs with a D-dimer level ≤ 0.5 µg/mL (81% vs 78%, *P* = .99). Twenty-three (48%) dogs received a doxorubicin-based chemotherapy protocol at the time of relapse, including 14 dogs with pretreatment D-dimer level ≤ 0.5 µg/mL and nine dogs with pretreatment D-dimer level > 0.5 µg/mL.

All dogs died from lymphoma or were euthanized because of poor quality of life secondary to disease progression. For the entire cohort, the median PFS was 86 days (range: 4-224 days) and the median OS was 121 days (range: 4-466 days). The median PFS for dogs with a pretreatment D-dimer level > 0.5 µg/mL was 54 days (range: 4-153 days) compared with 104 days (range: 10-224 days) for dogs with a D-dimer level ≤ 0.5 µg/mL (*P* = .011). For dogs with a pretreatment D-dimer level > 0.5 µg/mL, the median OS was 93 days (range: 4-280 days) compared with 169 days (range: 10-466 days) for dogs with a D-dimer level ≤ 0.5 µg/mL (*P* = .003). Kaplan-Meier curves are shown in Figure 1.

In the univariate analysis, dogs with pretreatment D-dimer level > 0.5 µg/mL were significantly more likely to have a shorter PFS compared with dogs with pretreatment D-dimer level ≤ 0.5 µg/mL (HR: 2.22, 95% CI: 1.18-4.19; *P* = .042, Table 3). Other parameter that predicted significantly shorter PFS in the univariate analysis included prior chemotherapy (*P* = .042). After post hoc correction, WHO stage V was not found to be significant (*P* = .066). In the multivariate Cox regression model, pretreatment D-dimer level > 0.5 µg/mL (HR: 3.21, 95% CI: 1.57-6.56, *P* = .001) and prior chemotherapy (HR: 5.06, 95% CI: 1.92-13.37, *P* = .001) remained independent predictors for worse PFS .

Univariate and multivariate analyses were also performed for OS (Table 4). The univariate analysis revealed that pretreatment plasma D-dimer level > 0.5 µg/mL (*P* = .024), prior chemotherapy (*P* = .027) and WHO stage V (*P* = .032) were significantly correlated to OS. The multivariate analysis confirmed that pretreatment plasma D-dimer level > 0.5 µg/mL (HR: 3.87, 95% CI: 1.88-7.98; *P* < .001), prior chemotherapy (HR: 9.71, 95% CI: 3.27-28.83; *P* < .001) and WHO stage V (HR: 3.13, 95% CI: 1.41-6.96; *P* = .005), were independent predictors of OS.

No significant difference in response rate (76% vs 83%, *P* = .73), PFS (HR: 0.73, 95% CI: 0.40-1.33; *P* = .37) and OS (HR: 0.83, 95% CI: 0.46-1.50; *P* = .64) was identified between dogs with intermediate to high-grade NHL treated with F14512 or etoposide phosphate.

4 | DISCUSSION

The results of this prospective study a provide strong evidence that elevation of pretreatment plasma D-dimer level is associated with worse outcome in dogs with intermediate to high-grade NHL. In the present study, pretreatment D-dimer level >0.5 µg/mL was an independent predictor for a shorter PFS and OS.

The relationship between D-dimer level and tumours has been the subject of recent attention. The exact mechanisms underlying the associated between the D-dimer levels and cancer survival remains to be elucidated. A probable explanation may be related with the activation of the coagulation-fibrinolysis system by the cancer cells.[38-40] The expression of tissue factors produced by the cancer cells, the

TABLE 3 Univariate and multivariate analysis of prognostic factors for progression-free survival

	Univariate analysis			Multivariate analysis	
Variable	HR (95% CI)	*P* value	FDR adjusted *P* value	HR (95% CI)	*P* value
WHO clinical stage (V vs II + III + IV)	2.75 (1.27-5.95)	0.011	0.066		
D-dimer level (> 0.5 vs ≤0.5 μg/mL)	2.22 (1.18–4.19)	0.014	0.042	3.21 (1.57–6.56)	.001
Prior chemotherapy (yes vs no)	2.71 (1.16-6.34)	0.021	0.042	5.06 (1.92-13.37)	.001
Immunophenotype (T vs B)	2.20 (0.93-5.19)	0.07	0.1		
Treatment group (F14512 vs Etoposide phosphate)	0.73 (0.40-1.33)	0.31	0.37		
WHO clinical substage (b vs a)	1.27 (0.71-2.26)	0.42	0.42		

Abbreviations: FDR, false discovery rate; WHO, World Health Organization.

TABLE 4 Univariate and multivariate analysis of prognostic factors for overall survival

	Univariate analysis			Multivariate analysis	
Variable	HR (95% CI)	*P* value	FDR adjusted *P* value	HR (95% CI)	*P* value
D-dimer level (>0.5 vs ≤0.5 μg/mL)	2.53 (1.34-4.78)	0.004	0.024	3.87 (1.88–7.98)	<.001
Prior chemotherapy (yes vs no)	3.33 (1.35-8.18)	0.009	0.027	9.71 (3.27-28.83)	<.001
WHO clinical stage (V vs II + III + IV)	2.51 (1.19-5.32)	0.016	0.032	3.13 (1.41-6.96)	.005
WHO clinical substage (b vs a)	1.43 (0.78-2.61)	0.24	0.36		
Treatment group (F14512 vs Etoposide phosphate)	0.83 (0.46-1.50)	0.53	0.64		
Immunophenotype (T vs B)	1.28 (0.56-2.92)	0.56	0.56		

Abbreviations: FDR, false discovery rate; WHO, World Health Organization.

activation of thrombin in tumour microenvironment and the secretion of proinflammatory factors result in activation of coagulation and plasminogen activators.[39-42] This abnormal activation of coagulation-fibrinolysis system eventually promotes tumour growth, tumour invasion and angiogenesis.[39] Elevated D-dimer level may reflect this abnormal coagulation-fibrinolysis activation and thereby elevated D-dimer is associated with worse outcome in cancer patients.

For the purpose of comparison, we compared dogs with a low (≤0.5 μg/mL) or high (>0.5 μg/mL) pretreatment plasma D-dimer level based on the published data of D-dimer reference range in clinically healthy dogs. In the present study, 44% (21/48) of dogs with intermediate to high-grade NHL had pretreatment plasma D-dimer level above 0.5 μg/mL. No significant correlations were found between pretreatment plasma D-dimer level and clinical features including sex, age, WHO tumour stage and substage, immunophenotype and treatment response. However, in humans malignant haematopoietic tumours, high pretreatment plasma D-dimer level correlates with adverse clinical profiles such as Ann-Arbor stage of III-IV, presence of b clinical signs, a worse Eastern Cooperative Oncology Group (ECOG) Performance Status, International Prognostic Index (IPI) score of intermediate and high risk and extra-nodal sites ≥2.[56] The small number of dogs included in the present study might explain the absence of significant correlation identified between pretreatment plasma D-dimer level and the clinical features in dogs and further studies are warranted to confirm these findings.

Based on a Cox proportional hazard model, our results revealed that high pretreatment plasma D-dimer level (>0.5 μg/mL) was a significant prognostic indicator on PFS and OS in dogs with intermediate to high-grade NHL and it was found to be an independent determinant of prognosis on multivariate analysis. Other previously described prognostic indicators on OS in dogs with lymphoma were also identified in our study, including prior chemotherapy and WHO stage V lymphoma. However, T-cell immunophenotype and WHO clinical substage b were not identified as a significant prognostic indicator. These results might be explained by the small number of dogs included. In the present study, pretreatment sTK1 activity was also evaluated. A low correlation was found between pretreatment plasma D-dimer level and sTK1 activity and pretreatment sTK1 activity was not correlated with PFS and OS. Our results suggest that sTK1 activity may not be used as a specific prognostic factor in dogs with intermediate to high-grade NHL.

There are some limitations concerning the present study. First, the prognostic value of pretreatment plasma D-dimer level was evaluated in a randomized double-blind study, assessing two innovative anti-neoplastic drugs (F14512 and etoposide phosphate). Pretreatment D-dimer level was not a determinative factor for treatment planning or treatment group assignment. Treatments received were balanced between the groups of dogs with pretreatment plasma D-dimer level >0.5 and ≤0.5 μg/mL. There was no difference in response rate, PFS or OS between dogs with intermediate to high-grade NHL

treated with F14512 or etoposide phosphate. The results of the present study would not have been expected to be significantly affected by the treatment received. However, a prospective study is warranted to confirm the predictive value of pretreatment plasma D-dimer level in dogs with intermediate to high-grade NHL treated with a standardized CHOP-based chemotherapy protocol. Another limitation of the study is that D-dimer level was measured only once, prior to treatment initiation. Future longitudinal studies with serial measurements of D-dimer levels would be necessary to evaluate the correlation with treatment response and disease progression. In addition, elevated D-dimer levels in human patients with malignant solid tumours were associated with a hypercoagulable state and increased risk of venous thromboembolism (VTE).[57,58] No dogs in our study experienced symptomatic VTE, indicating that VTE might not be the reason for poor survival reported in dogs with higher levels of D-dimer. However, asymptomatic VTE influencing the disease course and prognosis cannot be excluded. In addition, post-mortem examinations were not routinely performed, and vascular embolism may have been missed in some dogs, particularly in dogs with high pretreatment plasma D-dimer levels.

In conclusion, we herein identified high pretreatment plasma D-dimer level as a potential marker to predict survival in dogs with intermediate to high-grade NHL. These findings require further validation in dogs treated with a standardized CHOP-based chemotherapy protocol and employing a prospective study design.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

Ethical approval was obtained from the OCR Ethical Committee before study initiation.

DATA AVAILABILITY STATEMENT

All data associated with this study are available in the main text or the supplementary materials (Supporting Information Table S1).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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